EXHIBIT E

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Severe Growth Defect in a *Schizosaccharomyces pombe* Mutant Defective in Intron Lariat Degradation

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The cDNAs and genes encoding the intron lariat-debranching enzyme were isolated from the nematode *Caenorhabditis elegans* and the fission yeast *Schizosaccharomyces pombe* based on their homology with the *Saccharomyces cerevisiae* gene. The cDNAs were shown to be functional in an interspecific complementation experiment; they can complement an *S. cerevisiae dbr1* null mutant. About 2.5% of budding yeast *S. cerevisiae* genes have introns, and the accumulation of excised introns in a *dbr1* null mutant has little effect on cell growth. In contrast, many *S. pombe* genes contain introns, and often multiple introns per gene, so that *S. pombe* is estimated to contain ~40 times as many introns as *S. cerevisiae*. The *S. pombe dbr1* gene was disrupted and shown to be nonessential. Like the *S. cerevisiae* mutant, the *S. pombe* null mutant accumulated introns to high levels, indicating that intron lariat debranching represents a rate-limiting step in intron degradation in both species. Unlike the *S. cerevisiae* mutant, the *S. pombe dbr1::leu1*$^+$ mutant had a severe growth defect and exhibited an aberrant elongated cell shape in addition to an intron accumulation phenotype. The growth defect of the *S. pombe dbr1::leu1*$^+$ strain suggests that debranching activity is critical for efficient intron RNA degradation and that blocking this pathway interferes with cell growth.

Pre-mRNA introns are excised from precursor RNA in the form of a lariat RNA structure during the process of RNA splicing. In this structure, the 5′ end of the intron is joined via a 2′-5′ phosphodiester linkage to an internal adenosine (A) residue (the branchpoint nucleotide). These introns are excised by a two-step reaction (9, 25, 29). In the first step, the 5′ splice site is cleaved to yield a 5′ exon intermediate (exon 1). Concurrently, the 5′ end of the intron is joined to the branch site (usually an adenosine residue), forming a 2′-5′ phosphodiester bond. This forms the lariat intermediate RNA (or 2/3 intermediate) consisting of a lariat-form intron joined to the 3′ exon (exon 2). The exon 1 and lariat intron-exon 2 intermediates are subsequently resolved by a second cleavage reaction at the 3′ splice site which releases the mature lariat form of intron RNA and the ligation product of exons 1 and 2 via a 3′-5′ phosphodiester bond, the mature mRNA. The excised intron lariat RNA is rapidly degraded in vivo, with a half-life of only a few seconds (31). Thus, in general, the spliced introns are not sufficiently stable in eukaryotic cells to be detected in vivo.

In contrast to the splicing pathway leading from pre-mRNA to mRNA, very little is known about the pathway of intron degradation. Considering the enormous quantity of intron RNA that is discarded during the process of normal gene expression in mammalian cells, for example, intron degradation is likely to be an important pathway for normal cellular function. Only one component of the intron turnover pathway has been identified biochemically and genetically, the RNA lariat-debranching enzyme (DBR). DBR specifically hydrolyzes the 2′-5′ phosphodiester bond at the branchpoint of intron lariat RNAs, converting lariats into linear molecules (6, 15, 30). The linearized introns are subsequently degraded by undefined exonucleases.

Although RNA lariat-debranching enzymatic activity was originally identified from human HeLa cell extracts (30), the gene encoding budding yeast (*Saccharomyces cerevisiae*) debranching enzyme, *DBR1*, was identified and cloned by using a genetic screen aimed at identifying cellular factors involved in Ty1 retroelement transposition (3). The *S. cerevisiae dbr1* mutant strain reduces Ty1 transposition frequency and also has a severe defect in intron degradation, i.e., excised introns accumulate to very high levels in the form of "nibbled" lariat structures lacking their 3′ tails (6). Thus, debranching apparently represents a rate-limiting step in the intron degradation pathway. Somewhat unexpectedly, the *DBR1* gene can be disrupted with little effect on growth rate in *S. cerevisiae* (6).

DBR1 protein has been purified to near homogeneity from a bacterial overexpression strain (24). The enzyme is capable of digesting a variety of branched nucleic acid substrates, including lariats derived from pre-mRNA splicing and self-splicing group II introns, multicopy satellite DNAs, and a variety of synthetic branched RNAs (24).

In contrast to higher eukaryotes, which typically contain several large introns in most genes, relatively few *S. cerevisiae* genes contain introns, and they nearly always contain only a single intron (12, 27). Thus, it is not entirely surprising that *DBR1* is nonessential in *S. cerevisiae* and that the accumulation of excised introns has no deleterious effect on cell growth, i.e., the rapid turnover of excised introns is not essential in this species. In contrast, the efficient turnover of excised introns may be important or essential in more-complex eukaryotes. To examine this question, we have isolated the genes encoding DBR from two additional eukaryotes, *Caenorhabditis elegans* and *Schizosaccharomyces pombe*, organisms that both have much higher numbers of introns. The encoded proteins are homologous to the *S. cerevisiae* DBR protein and furthermore complement an *S. cerevisiae dbr1* null mutant strain. Interestingly, an *S. pombe dbr1* disruption mutant grew dramatically

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